REMARKS

Claims 1-21 are pending. Claims 1-8, 10, and 12-21 have been withdrawn from consideration. Claims 9 and 11 were rejected under 35 U.S.C. § 112, second paragraph, and under 35 U.S.C. § 102. Applicants address each rejection as follows.

Elected Claims

Applicants note that, while claims 1-21 were filed with the application, only claims 1-19 were placed in restriction groups in the Restriction Requirement mailed in this case on April 30, 2001. Since claims 20 and 21, like elected claims 9 and 11, are drawn to "a method for identifying a compound that modulates the biological activity of a serotonin-gated anion channel comprising administering a test compound and assaying a modulation" as stated in the Office's description of Group V (the elected group), Applicants submit that claims 20 and 21 are drawn to the elected invention. Accordingly, Applicants hereby request that claims 20 and 21 be examined in the present application.

Objection to the Specification

The Office objected to the specification for not including "a separate reference to and brief description of each of the drawing(s)." Applicants now amend the specification to refer to each drawing. Accordingly, this objection may be withdrawn. In addition, Applicants amend the specification to include sequence identifiers that are used throughout the specification and claims to refer to their respective sequences as submitted

in the Sequence Listing mailed on October 23, 2000. Furthermore, Applicants amend the brief description of Figure 4 to clearly describe the subject matter of this figure. No new matter has been added by these amendments.

Claim Amendments

Applicants amend claims 9 and 11 to clarify the claim language. Support for these amendments may be found on, for example, page 15, line 22, to page 16, line 5, page 16, lines 9-11, page 17, lines 2-5, page 33, line 12, to page 34, line 11, and page 39, lines 10-18. In addition, Applicants submit that SDS and SSC are standard acronyms in the art of molecular biology and, therefore, one skilled in this art would know that SDS refers to sodium dodecyl sulfate and that SSC is a solution of sodium chloride and sodium citrate (See, for example, Ausubel et al. Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1995, A.2.4 (copy enclosed)). Furthermore, Applicants add new claims 22-29. Support for claims 22 and 23 may be found, for example, at page 33, line 12, to page 34, line 11, of the specification and support for claims 24-29 may be found, for example, at page 17, lines 14-18, page 25, lines 13-18, and page 39, lines 10-18.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 9 and 11 were rejected under 35 U.S.C. § 112, second paragraph as being indefinite in failing to point out and distinctly claim the invention. Specifically, the Office asserts that claim 9 contains "no positive step indicating the correlation of a

change in biological activity with a compound's ability to modulate a serotonin-gated anion channel," and that "it is unclear what biological activity is being measured." Similarly, the Office asserts that claim 11 contains "no positive step indicating the correlation between modulation of activity associated with serotonin-gated anion channel and a specific characteristic of a drug" and that there is no antecedent basis for "the activity."

These objections have been overcome by the present clarifying amendments to claims 9 and 11. In particular, claims 9 and 11, as amended, refer to assaying or detecting a modulation in current flux, which can be accomplished using nothing more than methods standard in the art (see, for example, Applicants' specification at page 39, lines 10-18). In addition, Applicants note that the specification, for instance, at page 17, lines 2-13, provides examples of a variety of biological activities that one skilled in the art would know how to use to monitor the recited change, as well as numerous conditions associated with serotonin-mediated cellular responses (see, for example, page 36, lines 12-17). In view of these arguments, Applicants submit that present claims 9 and 11 particularly point out and distinctly claim the invention. Accordingly, the 35 U.S.C. § 112, second paragraph rejection of these claims may be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 9 and 11 were rejected under 35 U.S.C. § 102(a) as being anticipated by Scrogin et al. *Am. J. Physiol.* 275:R2035-R2042, 1998 ("Scrogin") and by Ali et al., *J.*

Physiol. 509:211-219, 1998 ("Ali"). Claims 9 and 11 were also rejected under 35 U.S.C. § 102(b) as being anticipated by De Montigny and Aghajanian, *Science* 202:1303-1306, 1978 ("De Montigny") and Garner et al., *Eur. J. Pharmacol.* 239:31-37, 1993 ("Garner").

Claim 9, as amended, is directed to a method for identifying a compound that modulates a biological activity of a serotonin-gated anion channel. This method involves administering a test compound to a cell with a serotonin-gated anion channel that is encoded by a purified nucleic acid sequence that hybridizes, under conditions comprising hybridization at about 42°C followed by a first wash at about 42°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 50°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, to a purified nucleic acid sequence including the sequence of SEQ ID NO:2, and assaying a modulation in current flux into or out of the cell. In addition, claim 11, as amended, is directed to a method for characterizing a drug as being associated with a serotonin-mediated cellular response. This method involves detecting a modulation in current flux through a serotonin-gated anion channel encoded by a purified nucleic acid sequence that hybridizes, under conditions comprising hybridization at about 42°C followed by a first wash at about 42°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 50°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, to a purified nucleic acid sequence including the sequence of SEQ ID NO:2, when this channel is exposed to the drug.

With regard to the references cited by the Office, Applicants note that none of these references teaches a serotonin-gated anion channel that is encoded by a purified nucleic acid sequence that hybridizes, under the conditions recited in the present claims, to a purified nucleic acid sequence including the sequence of SEQ ID NO:2. Since none of the cited references describes all the features of claims 9 or 11, these claims, and newly added claims 22-29 which depend from claim 9 or 11, cannot be anticipated by Scrogin, Ali, De Montigny, or Garner. Accordingly, the 35 U.S.C. § 102 rejections of claims 9 and 11 may be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance and such action is respectfully requested.

Enclosed are marked-up copies of the amended paragraphs and claims, as well as a clean copy of the amended paragraphs and of the amended and new claims.

Also enclosed is a petition to extend the period for replying for one month, to and including April 19, 2002. If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: April 19, 2002

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01997.521002 Reply to Examiner's Action dated 12.19.01.wpd

Version with Markings to Show Changes Made

Amend the paragraph beginning on page 20, line 16 as follows.

Fig. 1 shows the genomic sequence of *C. elegans mod-1* (SEQ ID NO:1).

Amend the paragraph beginning on page 20, line 17 as follows.

Fig. 2 shows the cDNA sequence encoding the *C. elegans* MOD-1 polypeptide (SEQ ID NO:2).

Amend the paragraph beginning on page 20, line 19 as follows.

Fig. 3 shows the *C. elegans* MOD-1 predicted amino acid sequence (SEQ ID NO:3).

Amend the paragraph beginning on page 20, line 20 as follows.

Fig. 4 shows the structure of the *C. elegans* [*mod-1* cDNA] MOD-1 amino acid sequence.

Amend the paragraph beginning on page 21, line 1 as follows

Fig. 6 shows the genomic sequence of the *C. elegans mod-1* gene with the ok103 mutation (SEQ ID NO:4).

Amend the paragraph beginning on page 21, line 3 as follows

Fig. 7 shows the genomic sequence of the *C. elegans mod-1* gene with the n3034 mutation (SEQ ID NO:5).

Amend the paragraph beginning on page 21, line 5 as follows

Fig. 8 shows the cDNA sequence of the *C. elegans mod-1* gene with the n3034 mutation (SEQ ID NO:6).

Amend the paragraph beginning at page 23, line 14 as follows.

The *mod-1* mutants, as described in the previous section, were further characterized using this technique. Animals carrying the n3034 mutation (Fig. 7 and Fig. 8) exhibited a dominant phenotype of insensitivity to exogenous serotonin in liquid locomotion assays. Animals carrying the ok103 mutation (Fig. 5 and Fig. 6) exhibited a recessive phenotype of insensitivity to exogenous serotonin in liquid locomotion assays.

Amend the paragraph beginning at page 23, line 22 as follows.

Both the wild type (Fig. 2), and mutant *mod-1* cDNA have been obtained. The dominant serotonin resistance phenotype of animals carrying the *mod-1(n3034)* allele was used to genetically map *mod-1(n3034)* to a 0.7 map-unit interval on chromosome V. Deficiency analysis showed that the dominant serotonin resistance phenotype is not due to a haploinsufficiency of the *mod-1* locus. The recessive nature of the serotonin resistance phenotype at early time points was exploited to perform standard transformation rescue experiments, and subsequently, the gene was cloned (Fig. 1).

Amend the paragraph beginning at page 24, line 7 as follows.

The protein encoded by the *mod-1* open reading frame responsible for the rescue is structurally similar to ligand-gated ion channels that belong to the nicotinic acetylcholine receptor (nAchR) family (Fig. 3 and Fig. 4). The nAchR family members are all pentameric channels with large N-terminal extracellular ligand-binding domains, four highly conserved transmembrane domains (M1-M4), and relatively divergent cytoplasmic domains between M3 and M4. nAchR family members include channels gated by acetylcholine, glycine, GABA, avermectin, and serotonin. Within the members of the nAchR family, structure-function analysis has been performed primarily on the acetylcholine receptor, but many structural and functional parallels have been seen with the other family members as well. In addition, chimeric channel studies show that there is a great deal of conservation at the functional level, even across the different ligand-

gated members of the family. The M2 domains of the various subunits are predicted to line the pore of the channels. Site-directed mutagenesis studies of residues within this domain have demonstrated that ion specificity and modulation of the magnitude and frequency of current flux are determined, at least in part, by the residues that line the pore and those that are immediately adjacent to the pore on both the extracellular and cytoplasmic sides. Based on primary sequence analysis, MOD-1 appears to be equally divergent from all cloned nAchR family members.

Amend the paragraph beginning at page 25, line 3 as follows.

MOD-1 was heterologously expressed in *Xenopus* oocytes, injected with 50 nl of *C. elegans* RNA, or MOD-1 was expressed in HEK cells transiently transfected by calcium phosphate precipitation. Forty-eight to 72 hours later, the cells or oocytes were screened under a voltage clamp (Figs. 9A-9C). Application of 100 nM serotonin elicited large inward currents at a holding potential of -70 mV. Uninjected oocytes and nontransfected cells had no response to 10 μM serotonin. Application of 1 mM of other agonists of ligand-gated ion channels, such as acetylcholine, GABA, or glycine elicited little or no response from the MOD-1 channel.

Amend the paragraph beginning at page 25, line 13 as follows.

Ion selectivity was determined by measuring changes in the reversal potential (voltage at which the serotonin response changes from an inward, negative, to an outward, positive, current) in response to varying the ionic composition of the bath solution. The reversal potential was insensitive to changes in cations (Na^+ or K^+), but shifted by approximately 50 mV for each 10-fold change in extracellular chloride concentration (Fig. 10).

Amend claims 9 and 11 as follows.

9. (Amended) A method for identifying a compound that modulates a biological

activity of a serotonin-gated anion channel, said method comprising the steps of:

(a) administering a test compound to a cell comprising a serotonin-gated anion channel encoded by a purified nucleic acid sequence that hybridizes, under conditions comprising hybridization at about 42°C followed by a first wash at about 42°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 50°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2; and

(b) assaying a modulation in [the] current flux into or out of said cell, wherein said modulation in current flux is indicative of a compound that modulates said biological activity of said serotonin-gated anion channel.

11. (Amended) A method for characterizing a drug as being associated with a serotonin-mediated cellular response, said method comprising detecting a modulation in [the activity of] current flux through a serotonin-gated anion channel encoded by a purified nucleic acid sequence that hybridizes, under conditions comprising hybridization at about 42°C followed by a first wash at about 42°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 50°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2, when said channel is exposed to said drug, wherein said modulation in current flux is indicative of said drug being associated with a serotonin-mediated cellular response.

Add new claims 22-29.

22. (New) The method of claim 9, wherein said purified nucleic acid sequence hybridizes to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2

under conditions comprising hybridization at about 42°C in about 50% formamide followed by a first wash at about 65°C in about 2X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 65°C in about 1X SSC sodium chloride/sodium citrate solution and about 0.1% Sodium Dodecyl Sulfate.

23. (New) The method of claim 11, wherein said purified nucleic acid sequence hybridizes to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2 under conditions comprising hybridization at about 42°C in about 50% formamide followed by a first wash at about 65°C in about 2X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 65°C in about 1X SSC sodium chloride/sodium citrate solution and about 0.1% Sodium Dodecyl Sulfate.

24. (New) The method of claim 9, wherein said modulation in current flux is a decrease in current flux.

25. (New) The method of claim 9, wherein said modulation in current flux is an increase in current flux.

26. (New) The method of claim 9, wherein said current flux comprises chloride ions.

27. (New) The method of claim 11, wherein said modulation in current flux is a decrease in current flux.

28. (New) The method of claim 11, wherein said modulation in current flux is an increase in current flux.

29. (New) The method of claim 11, wherein said current flux comprises chloride ions.

Clean Version of the Amended Paragraphs and Amended and New Claims

Fig. 1 shows the genomic sequence of *C. elegans mod-1* (SEQ ID NO:1).

Fig. 2 shows the cDNA sequence encoding the *C. elegans* MOD-1 polypeptide (SEQ ID NO:2).

Fig. 3 shows the *C. elegans* MOD-1 predicted amino acid sequence (SEQ ID NO:3).

Fig. 4 shows the structure of the *C. elegans* MOD-1 amino acid sequence.

Fig. 6 shows the genomic sequence of the *C. elegans mod-1* gene with the ok103 mutation (SEQ ID NO:4).

Fig. 7 shows the genomic sequence of the *C. elegans mod-1* gene with the n3034 mutation (SEQ ID NO:5).

Fig. 8 shows the cDNA sequence of the *C. elegans mod-1* gene with the n3034 mutation (SEQ ID NO:6).

The *mod-1* mutants, as described in the previous section, were further characterized using this technique. Animals carrying the n3034 mutation (Fig. 7 and Fig. 8) exhibited a dominant phenotype of insensitivity to exogenous serotonin in liquid locomotion assays. Animals carrying the ok103 mutation (Fig. 5 and Fig. 6) exhibited a recessive phenotype of insensitivity to exogenous serotonin in liquid locomotion assays.

Both the wild type (Fig. 2), and mutant *mod-1* cDNA have been obtained. The dominant serotonin resistance phenotype of animals carrying the *mod-1(n3034)* allele was used to genetically map *mod-1(n3034)* to a 0.7 map-unit interval on chromosome V. Deficiency analysis showed that the dominant serotonin resistance phenotype is not due to a haploinsufficiency of the *mod-1* locus. The recessive nature of the serotonin resistance phenotype at early time points was exploited to perform standard transformation rescue experiments, and subsequently, the gene was cloned (Fig. 1).

The protein encoded by the *mod-1* open reading frame responsible for the rescue is structurally similar to ligand-gated ion channels that belong to the nicotinic acetylcholine receptor (nAchR) family (Fig. 3 and Fig. 4). The nAchR family members are all pentameric channels with large N-terminal extracellular ligand-binding domains, four highly conserved transmembrane domains (M1-M4), and relatively divergent cytoplasmic domains between M3 and M4. nAchR family members include channels gated by acetylcholine, glycine, GABA, avermectin, and serotonin. Within the members of the nAchR family, structure-function analysis has been performed primarily on the acetylcholine receptor, but many structural and functional parallels have been seen with the other family members as well. In addition, chimeric channel studies show that there is a great deal of conservation at the functional level, even across the different ligand-gated members of the family. The M2 domains of the various subunits are predicted to line the pore of the channels. Site-directed mutagenesis studies of residues within this domain have demonstrated that ion specificity and modulation of the magnitude and frequency of current flux are determined, at least in part, by the residues that line the pore and those that are immediately adjacent to the pore on both the extracellular and cytoplasmic sides. Based on primary sequence analysis, MOD-1 appears to be equally divergent from all cloned nAchR family members.

MOD-1 was heterologously expressed in *Xenopus* oocytes, injected with 50 nl of

C. elegans RNA, or MOD-1 was expressed in HEK cells transiently transfected by calcium phosphate precipitation. Forty-eight to 72 hours later, the cells or oocytes were screened under a voltage clamp (Figs. 9A-9C). Application of 100 nM serotonin elicited large inward currents at a holding potential of -70 mV. Uninjected oocytes and nontransfected cells had no response to 10 μM serotonin. Application of 1 mM of other agonists of ligand-gated ion channels, such as acetylcholine, GABA, or glycine elicited little or no response from the MOD-1 channel.

Ion selectivity was determined by measuring changes in the reversal potential (voltage at which the serotonin response changes from an inward, negative, to an outward, positive, current) in response to varying the ionic composition of the bath solution. The reversal potential was insensitive to changes in cations (Na^+ or K^+), but shifted by approximately 50 mV for each 10-fold change in extracellular chloride concentration (Fig. 10).

9. (Amended) A method for identifying a compound that modulates a biological activity of a serotonin-gated anion channel, said method comprising the steps of:

(a) administering a test compound to a cell comprising a serotonin-gated anion channel encoded by a purified nucleic acid sequence that hybridizes, under conditions comprising hybridization at about 42°C followed by a first wash at about 42°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 50°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2; and

(b) assaying a modulation in current flux into or out of said cell, wherein said modulation in current flux is indicative of a compound that modulates said biological activity of said serotonin-gated anion channel.

11. (Amended) A method for characterizing a drug as being associated with a serotonin-mediated cellular response, said method comprising detecting a modulation in current flux through a serotonin-gated anion channel encoded by a purified nucleic acid sequence that hybridizes, under conditions comprising hybridization at about 42°C followed by a first wash at about 42°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 50°C in about 6X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2, when said channel is exposed to said drug, wherein said modulation in current flux is indicative of said drug being associated with a serotonin-mediated cellular response.

22. (New) The method of claim 9, wherein said purified nucleic acid sequence hybridizes to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2 under conditions comprising hybridization at about 42°C in about 50% formamide followed by a first wash at about 65°C in about 2X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 65°C in about 1X SSC sodium chloride/sodium citrate solution and about 0.1% Sodium Dodecyl Sulfate.

23. (New) The method of claim 11, wherein said purified nucleic acid sequence hybridizes to a purified nucleic acid sequence comprising the sequence of SEQ ID NO:2 under conditions comprising hybridization at about 42°C in about 50% formamide followed by a first wash at about 65°C in about 2X SSC sodium chloride/sodium citrate solution and about 1% Sodium Dodecyl Sulfate, and a second wash at about 65°C in about 1X SSC sodium chloride/sodium citrate solution and about 0.1% Sodium Dodecyl Sulfate.

24. (New) The method of claim 9, wherein said modulation in current flux is a

decrease in current flux.

25. (New) The method of claim 9, wherein said modulation in current flux is an increase in current flux.

26. (New) The method of claim 9, wherein said current flux comprises chloride ions.

27. (New) The method of claim 11, wherein said modulation in current flux is a decrease in current flux.

28. (New) The method of claim 11, wherein said modulation in current flux is an increase in current flux.

29. (New) The method of claim 11, wherein said current flux comprises chloride ions.

Table A.2.2 Preparation of 0.1 M Sodium and Potassium Acetate Buffers[a]

Desired pH	Solution A (ml)	Solution B (ml)
3.6	46.3	3.7
3.8	44.0	6.0
4.0	41.0	9.0
4.2	36.8	13.2
4.4	30.5	19.5
4.6	25.5	24.5
4.8	20.0	30.0
5.0	14.8	35.2
5.2	10.5	39.5
5.4	8.8	41.2
5.6	4.8	45.2

[a]Adapted by permission from CRC, 1975.

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate$\cdot 2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

Table A.2.3 Preparation of 0.1 M Sodium and Potassium Phosphate Buffers[a]

Desired pH	Solution A (ml)	Solution B (ml)	Desired pH	Solution A (ml)	Solution B (ml)
5.7	93.5	6.5	6.9	45.0	55.0
5.8	92.0	8.0	7.0	39.0	61.0
5.9	90.0	10.0	7.1	33.0	67.0
6.0	87.7	12.3	7.2	28.0	72.0
6.1	85.0	15.0	7.3	23.0	77.0
6.2	81.5	18.5	7.4	19.0	81.0
6.3	77.5	22.5	7.5	16.0	84.0
6.4	73.5	26.5	7.6	13.0	87.0
6.5	68.5	31.5	7.7	10.5	90.5
6.6	62.5	37.5	7.8	8.5	91.5
6.7	56.5	43.5	7.9	7.0	93.0
6.8	51.0	49.0	8.0	5.3	94.7

[a]Adapted by permission from CRC, 1975.